



Thanatochemical Utility of Postmortem Vitreous Biochemical Parameters in the Discrimination of Deaths Connected with Bleach Toxicity using Animal Models

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Abstract

The vitreous, unlike blood which putrefies shortly after death, promises to be a viable alternative specimen in the chemistry of death but there are no vitreous-based biomarkers for distinguishing amongst the emerging common causes of deaths. This has created a diagnostic gap that is being exploited to mask homicide as the court finds it difficult to accurately ascertain the cause of death where there are no physical injuries, as in cases of chemical toxicities. This study aims at exploring the discriminatory potentials of selected vitreous biochemical parameters in the autopsy of death by bleach toxicity and in death disguised as bleach toxicity using rabbits. Completely randomized block design (CRBD) was used for this study. 96 male rabbits were used for this research and were structured into four groups of twenty-four rabbits each: two treatment (test) groups and two control groups. In one test group, the death of the experimental subjects was caused by bleach toxicity. In the second test group, the death of the experimental subjects was caused by strangulation, thereafter, the dead subjects were administered lethal doses of bleach as a cover up of the actual cause of death. The remaining two groups are the baseline controls. After a postmortem interval of twenty-four hours, vitreous samples were obtained from each group of the experimental animals by Coe and Tente's methods. The samples were analyzed for the levels of Sodium, Potassium, Carbon iv oxide, Chloride and Calcium using ion selective electrode method, while Total protein, Albumin, Globulin, Glucose, Total cholesterol, Triacylglycerol, Urea, Creatinine, Uric acid, Creatine kinase and Lactate dehydrogenase were analyzed using their specific standard methods. Results obtained were then analyzed with the Statistical Package for Social Sciences (Version 18-21) and one-way ANOVA (Post Hoc-LSD). The results show that postmortem vitreous levels of Na, K, Cl, total protein, creatinine and lactate dehydrogenase differed significantly ($P \leq 0.05$) between samples from deaths

caused by bleach toxicity and deaths by strangulation but disguised as bleach toxicity. The striking differences in the levels of these notable analytes can be utilized either as primary or confirmatory tests to reveal and discriminate between death disguised as bleach toxicity and death by actual bleach toxicity.

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1.0 Introduction

Reports of the use of bleach for the purpose of committing suicides are on the increase. This is because bleach is a household cleaning substance that is unregulated and easily accessible (Slaughter *et al.*, 2019). However, discriminating between deaths caused by exposure to bleach through the oral route and postmortem administration of bleach in an attempt to cover-up extrajudicial killing has been almost impossible due to the absence of specific biomarkers that can aid such diagnosis (Martz *et al.*, 2022). Sodium hypochlorite (NaOCl), the main compound in bleach, when ingested instantly induces acute toxicity that brings about death within minutes. The adverse effects of sodium hypochlorite are diverse but include respiratory and metabolic acidosis, inflammation and perforation of the esophagus, pharynx, mediastinum, sepsis, hypothermia and kidney failure (Hifumi *et al.*, 2010; Park *et al.*, 2011).

Bleach, a caustic chemical, is an extremely useful disinfectant and potentially dangerous substance that has been utilized in medicine and by industry since the 18th century (Peck *et al.*, 2011). NaOCl is a chemical compound consisting of sodium, oxygen and chlorine. It is manufactured by the bubbling of chlorine gas into sodium hydroxide (NaOH) to form equal amounts of NaOCl and NaCl. NaOCl has

extremely low toxicity in the concentrations (0.45–0.5%) used for medical disinfection. At high concentrations non-diluted bleach is highly toxic. Commercial bleach products concentration ranges between 3-5% with an approximate pH of 11 (Bruch, 2007).

When bleach is being ingested, it elicits severe toxic effects. Sodium hypochlorite is a very corrosive chemical. Diluted sodium hypochlorite generally causes mild and minor damage to the esophagus, causing first-degree burns with hyperemia, edema of the mucosa and stomach irritation (Peck *et al.*, 2011). Ingesting large amounts of bleach results in severe symptoms because industrial strength bleach contains much higher concentrations of sodium hypochlorite (Arevalo-Silva *et al.*, 2006). Large volume ingestion of bleach is strongly associated with murder and suicide attempts, this result in disastrous complications such as severe esophageal burns with perforated gastroesophageal junction, bilateral pneumothorax and pneumoperitoneum and subsequent death (Ross and Spiller, 1999). Respiratory tract damage is present in cases of bleach ingestion. The damage can be as a result of inhaled vapors of bleach or aspiration during ingestion or vomiting after intake of chemical (Kuiper *et al.*, 2005). In cases of perforation, the contaminated esophageal mucosa is opened into the mediastinum, the thorax cavity that allows

passage of oral bacteria. Which results in systemic inflammatory response syndrome (SIRS), sepsis or eventually septic shock, acute renal failure caused by sepsis is at an average of 70% (David *et al.*, 2001). A study carried out by Schrier and Wang (2004) reported that diffuse systemic vasodilatation results in poor renal perfusion and acute kidney injury. Acute renal failure occurs in 19% of patients with moderate sepsis and up to 51% of patients with septic shock.

Blood, the usual analytical specimen, is unsuitable as a sample when an autopsy is conducted long after death because it decomposes shortly after death. This far-reaching diagnostic gap of making a distinction between an actual cause of death and a purported cause of death is being exploited by murderers to evade justice while homicidal crimes are on the rise. Some reasons why vitreous humour would make a good sample for postmortem forensic analyses are: unlike blood, it is not degraded for a long period after death (Adam and Gail, 2013); it undergoes very slow postmortem changes (Thierauf *et al.*, 2011); it contains several molecules and metabolites that can be assayed for or monitored (Amith, 2005); it is present in sufficient quantities that can serve as samples for multiple investigations (Garg *et al.*, 2004); it is easy to obtain (Zilg *et al.*, 2009).

The aim of this study is to explore the potentials of selected vitreous biochemical parameters as biomarkers in postmortem determination and discrimination of deaths by bleach toxicity using animal models. The objective of this is to measure and compare the postmortem vitreous levels of Sodium, Potassium, Carbon iv oxide, Chloride, Calcium, Total protein, Albumin, Globulin, Glucose, Total cholesterol, Triacylglycerol, Urea, Creatinine, Uric acid, Creatine kinase and Lactate dehydrogenase of dead experimental subjects in different groups based on differences in the causes of death in order to identify analytes with significant

variations in quantities amongst the studied groups that can be used as biomarkers for making distinctions amongst them.

The findings of this study may uncover unexplored facts valuable for developing biomarkers to distinguish between death disguised as bleach toxicity and death caused by actual bleach toxicity. Additionally, it aims to explore the potential of vitreous biochemical parameters in postmortem investigations, providing an alternative to blood samples for determining causes of death. This research could lead to the development of novel techniques or methodologies to corroborate existing approaches in postmortem analyses, thereby enhancing forensic investigations. Furthermore, it has the potential to improve the justice delivery system by offering critical information to resolve controversial cases involving bleach toxicity. By providing novel insights, it may contribute to reducing murder crimes in society by addressing how perpetrators escape punishment in homicides disguised as suicidal bleach toxicity. Finally, this study is expected to generate data that will guide future research on scientific solutions to postmortem legal challenges.

2.0 Materials and methods

2.1 Study area

This study was conducted at Eni-yimini Laboratories (eL) Ltd, Yenagoa, Bayelsa State. Yenagoa is an urban town that serves as the capital of the Yenagoa Local Government Area and also the Capital of Bayelsa State. It is located in the oil-rich Niger Delta region of Southern Nigeria and its geographical coordinates are 4⁰55'29"N and 6⁰15'51"E. It has a tropical monsoon climate with temperature fluctuations between 71⁰F and 87⁰F. Its weather vacillates between a wet rainy season and a cloudy dry season. Its vegetation is composed of four ecological zones – coastal barrier island forests, mangrove forests, freshwater swamps and lowland rain forests. Apart from being a

Government Administrative Metropolis and a Trade Centre for Agricultural produce, petroleum exploitation activities within its surroundings have introduced some Vocational diversifications with associated commercial and technological advances (Agoro *et al.*, 2021).

2.2 Animal specimen and study population

The research utilized 96 male albino rabbits. The age range was between six to eight months. The weight bracket/range was between 1.5 to 2kg. The animals were obtained from the animal house of the University of Jos, Plateau State. The animals were kept in cages at the animal house in the Biochemistry laboratory of the Federal University Otuoke, Bayelsa State, for 7 days prior to the experiment to allow for acclimatization to the environmental condition at room temperature. Commercial rat pellets and water were provided *ad libitum*.

Mead's resource equation was utilized for the calculation of the sample size (Kirkwood and Robert, 2010). The equation is stated and the components defined. $E = N - B - T$, where N is the total number of individuals or units in the study (minus 1). B is the blocking component, representing environmental effects allowed for in the design (minus 1). T is the treatment component, corresponding to the number of treatment groups (including the control group) being used or the number of questions being asked (minus 1). E is the degrees of freedom of the error component, and should be somewhere between 10 and 20. The study consisted of four groups ($T = 4$), with 24 animals per group, making 96 animals in total ($N = 95$), without any further stratification ($B = 0$), then E would equal 91, which is above the cutoff of 20, indicating that the sample size is very suitable for the research.

2.3 Experimental design

The research was structured into four groups of twenty-four rabbits each: two treatment (test) groups and two control groups based on specific means of deaths. Each group was administered a

unique treatment in the form of cause of death. In one test group, the death of the experimental subjects was caused by bleach toxicity. In a second test group, the death of the experimental subjects was caused by strangulation, thereafter, the dead subjects were administered lethal doses of bleach as a disguise or cover up of the actual cause of death. The remaining two groups are the baseline controls, which were deaths by strangulation and deaths by chloroform intoxication. Acute bleach toxicity was implemented by the administration of a bleach volume of 10 ml per body weight in kg orally, while strangulation was done by hand: the thumbs of both hands were pressed directly against the neck of the experimental subject forcefully and firmly, reflecting how most human homicides are undertaken. The observed average agonal periods were 23mins in bleach treatments, 10mins in strangulation and 27mins in chloroform treatment. Ascertaining the death status of the experimental animals relied on the prescriptions of the uniform determination of death act (Omelianchuk *et al.* 2022). Subjects in both treatment and control groups were left for 24 hours after death before their vitreous humour samples were collected for analysis, mimicking a scenario of death cover-up and subsequent discovery. The choice of vitreous is based on its long postmortem interval before deterioration, fermentation and putrefaction. In addition to its similarity in biochemical concentrations to blood, coupled with insignificant age and sex influences (Agoro *et al.*, 2018, 2019, 2020). Rabbit was the choice animal model for this research work. The suitability of rabbit as a choice animal for this study is attributed to its anatomical and physiological similarities to human (Gwon, 2008). Treatments and sample collections were conducted at the Biochemistry laboratory of the Federal University Otuoke, Bayelsa State. However, the sample analysis was conducted at the Eni-yimini Laboratories (eL) Ltd, located in Igbogene, Yenagoa, Bayelsa State.

2.4 Selection criteria

Rabbits used were apparently healthy and active as confirmed and approved by a veterinary doctor. Rabbits showing signs and symptoms of illness were excluded from the research. Also excluded were rabbits with any form of derangements. Turbid vitreous humours were rejected.

2.5 Collection of samples

The vitreous humour samples were collected by the method of Coe (1993) and Tente (2004). Briefly, using a 5 mL syringe and a needle, a scleral puncture was made on the lateral canthus and the total extractable vitreous humour was aspirated from the eye. Adequate care was taken to gently aspirate the fluid to avoid tearing of any loose tissue fragments surrounding the vitreous chamber. On average, 1.0 mL was collected from each rabbit's eye. Only crystal-clear liquid free of tissue contaminants and fragments was used in the study. The samples were collected twenty-four hours postmortem.

2.6 Preparation of samples

Collected vitreous humour samples meant for glucose concentration measurements were transferred into fluoride oxalate tubes, while those designated for investigations involving proteins, electrolyte, uric acid, lipid profile, renal and cardiac biomarkers were transferred into plain containers. The biochemical analyses were carried out immediately after extraction. Prior to analysis the vitreous samples were centrifuged at 2050 rpm for 10 min. The supernatants were separated and used for the analyses. The samples were employed for determination of the concentrations of sodium, potassium, chloride, calcium, bicarbonate, total protein, albumin, globulin, urea, creatinine, glucose, cholesterol, triacylglycerol, uric acid, lactate dehydrogenase (LDH) and creatine kinase (CK) (Agoro *et al*, 2021).

2.7 Analysis of samples

The selected vitreous electrolytes concentrations were determined using an ion selective electrode

(ISE) analyzer method (analyzer ISE 4000) as stated by Bolarin and Azinge (2010). Vitreous total protein concentration was estimated quantitatively using the Biuret Method as modified by Randox Laboratories (United Kingdom) (Randox kit leaflet). Biuret method is the most widely used method for protein analysis in body fluids (Peters, 1996) and one recommended by the International Federation of Clinical Chemistry (IFCC) expert panel for the determination of total protein (Randox kit leaflet). Vitreous albumin was estimated quantitatively using the Bromocresol Green Method as modified by Randox Laboratories (United Kingdom) (Randox kit leaflet). Vitreous globulin concentration was derived by subtracting vitreous albumin from vitreous total protein. The value is an estimate of vitreous globulin. Vitreous urea was estimated by the Diacetyl monoxime method. The diacetyl monoxime salts are stable at room temperature and could adapt to harsh environments (Bolarin and Azinge, 2010). Determination of vitreous creatinine concentration was by Jaffe's method. It was chosen because of its stability and consistency (Bolarin and Azinge, 2010). Vitreous glucose concentration was estimated quantitatively using the Glucose oxidase method as specified by Randox Laboratories (United Kingdom). The glucose kit used was a product of Randox and the standard operating procedure as stated in the manual was strictly followed. Vitreous total cholesterol concentration was estimated quantitatively using Agappe kit as specified by Agappe Diagnostics (Switzerland) (Agappe Kit Leaflet). Vitreous triacylglycerol concentration was estimated quantitatively using the Agappe kit as specified by Agappe Diagnostics (Switzerland) (Agappe Kit Leaflet). Vitreous uric acid concentration was estimated quantitatively by the Uricase Method using Agappe kit as specified by Agappe Diagnostics (Switzerland) (Agappe Kit Leaflet). Vitreous creatine kinase activity was estimated quantitatively using the Agappe method as

modified by Agappe Diagnostics (Switzerland) (Agappe Kit Leaflet). Vitreous lactate dehydrogenase (LDH) activity was estimated quantitatively using the Agappe method as modified by Agappe diagnostics (Switzerland) (Agappe kit leaflet) (Ikimi *et al.*, 2023; Ikimi *et al.*, 2024).

2.8 Statistical analyses

Each parameter quantity in the samples was determined in triplicates and the data generated were analyzed with the Statistical Package for Social Sciences (SPSS) program (SPSS Inc., Chicago, IL, USA; Version 18-21) and Microsoft Excel. One-way Analysis of variance (ANOVA) (Post Hoc-LSD) and student T-test were used in comparing the mean levels of the various vitreous parameters amongst the studied groups. The level of significance was considered at $P < 0.05$. Pearson correlation was used to determine relationships amongst the data generated.

3.0 Results and Discussion

The results of the levels of selected studied parameters in the postmortem vitreous of rabbits in cases of death by bleach toxicity and disguised as bleach toxicity are shown in Figs. 1 to 12. The results show that Na levels in BD (94.000 ± 0.40825 mmol/l) was significantly lower than in DBD (157.000 ± 0.40825 mmol/l) and in the controls (139.000 ± 0.040825 mmol/l) (145.000 ± 0.040825 mmol/l) respectively ($P \leq 0.05$) (Fig. 1). K level in BD (21.900 ± 0.04082 mmol/l) were significantly lower than in DBD (38.700 ± 0.04082 mmol/l) and in control 1 (38.300 ± 0.04082 mmol/l) (Fig. 2). Cl level in BD (71.000 ± 0.040825 mmol/l) was significantly lower than in DBD ($105.000 \pm$

0.40825 mmol/l) and in the controls (101.000 ± 0.47871 mmol/l) (99.000 ± 0.40825 mmol/l) respectively (Fig. 3). CO_2 levels were not significantly different in the test groups (BD and DBD) (14.000 ± 0.40825 mmol/l) (15.000 ± 0.40825 mmol/l) respectively but were significantly lower in control 1 (10.000 ± 0.47871 mmol/l) but not control 2 (16.000 ± 0.40825 mmol/l) (Fig. 4). Total protein level in DBD (13.488 ± 0.00041 mmol/l) was significantly higher than in BD (8.974 ± 0.00041 mmol/l) and in the controls (9.021 ± 0.00041 mmol/l) (4.613 ± 0.00041 mmol/l) respectively (Fig. 6). Calcium, albumin, glucose, total cholesterol, urea and uric acid levels were not statistically different in all groups (Fig. 5, 7 and 9). Globulin levels showed the same pattern in values with that of total protein in all groups (Fig. 8). Triacylglycerol levels in the test groups (1.037 ± 0.000 mmol/l) and (1.870 ± 0.19578 mmol/l) respectively were significantly different from the controls (0.010 ± 0.000 mmol/l) and (0.311 ± 0.000 mmol/l) respectively. Creatinine levels in DBD (42.511 ± 0.00041 mcmol/l) was significantly lower than in BD (60.932 ± 0.00041 mcmol/l) and in control 1 (83.002 ± 0.00041 mcmol/l) but lower in control 2 (28.663 ± 0.00041 mcmol/l) (Fig. 10). Creatine kinase activity in BD (3468.853 ± 0.00041 U/L) was significantly lower than in control 2 (5035.685 ± 0.00041 U/L) (Fig. 11). Lactate dehydrogenase activity in BD (13390.890 ± 0.00479 U/L) was significantly higher than in DBD (7265.726 ± 0.00041 U/L) and in the controls (17491.880 ± 0.00408 U/L) (9948.605 ± 0.000479 U/L) but lower than in control 1 (17491.880 ± 0.00408 U/L) (Fig. 12).

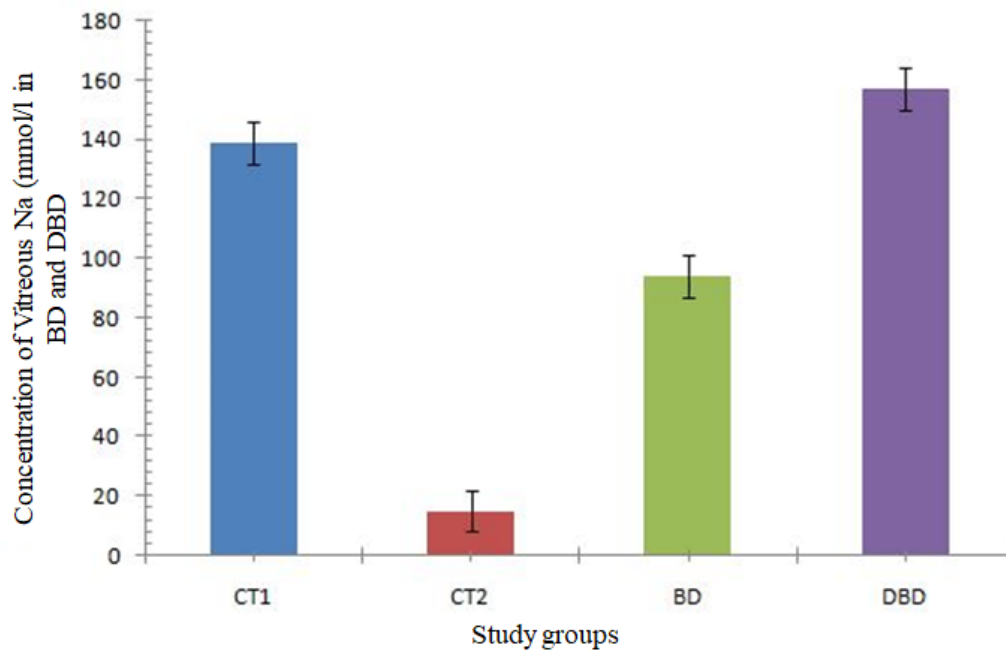


Fig. 1: Chart showing postmortem vitreous levels of Na in BD and DBD. CT1: Control 1 (Strangulation death); CT2: Control 2 (Chloroform death); BD: Bleach administration death; DBD: Disguised bleach death (strangled to death before bleach administration)

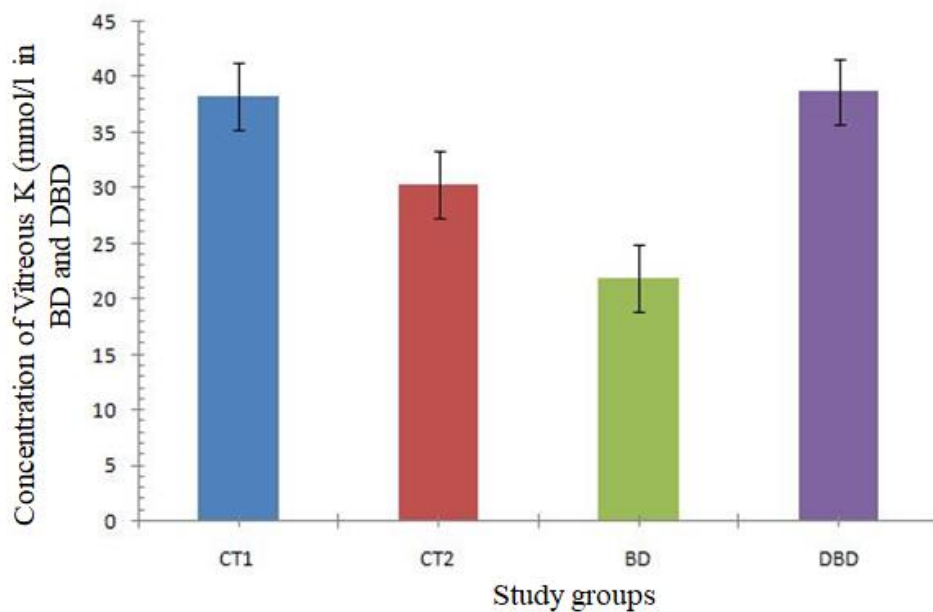


Fig. 2: Chart showing postmortem vitreous levels of K in BD and DBD. CT1: Control 1 (Strangulation death); CT2: Control 2 (Chloroform death); BD: Bleach administration death; DBD: Disguised bleach death (strangled to death before bleach administration)

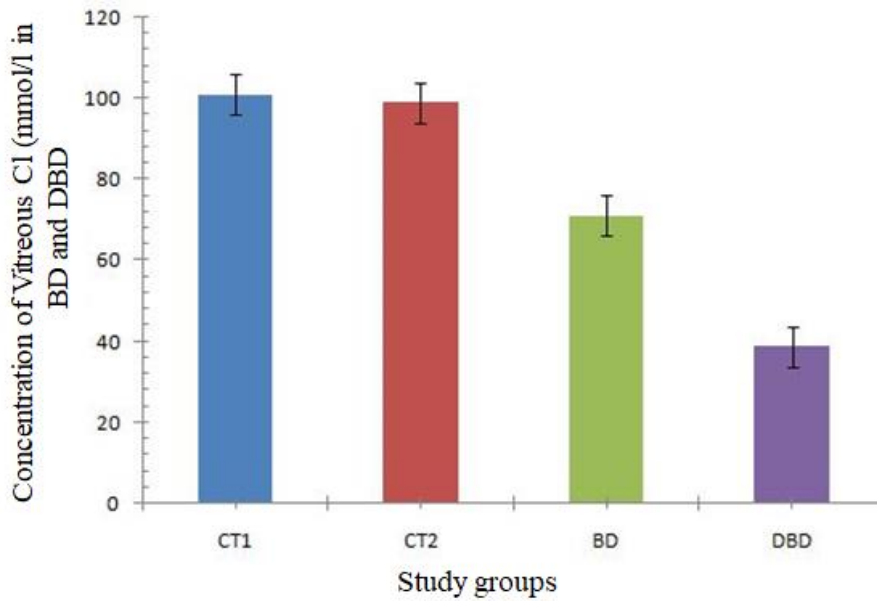


Fig. 3: Chart showing postmortem vitreous levels of Cl⁻ in BD and DBD. CT1: Control 1 (Strangulation death); CT2: Control 2 (Chloroform death); BD: Bleach administration death; DBD: Disguised bleach death (strangled to death before bleach administration)

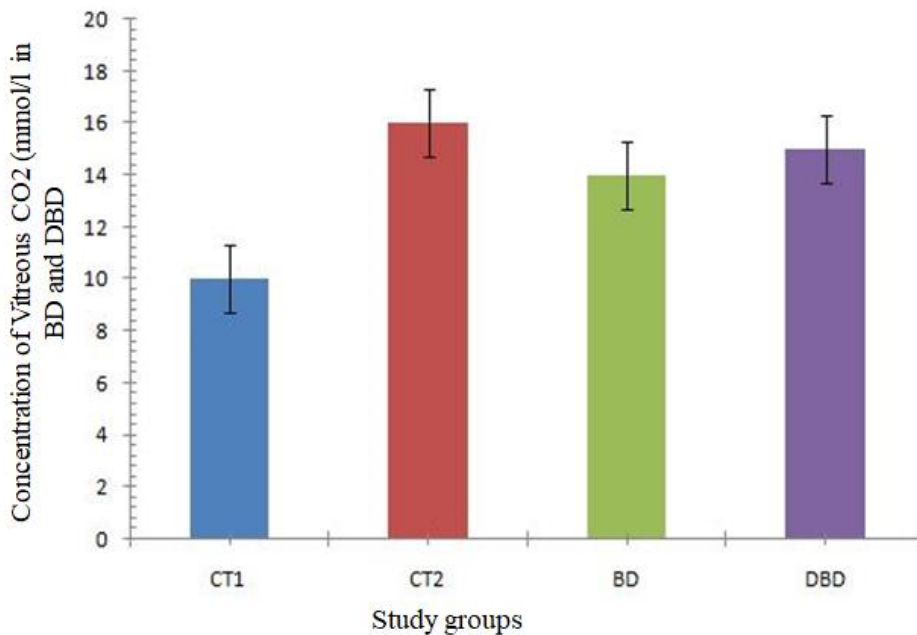


Fig. 4: Chart showing postmortem vitreous levels of CO₂ in BD and DBD. CT1: Control 1 (Strangulation death); CT2: Control 2 (Chloroform death); BD: Bleach administration death; DBD: Disguised bleach death (strangled to death before bleach administration)

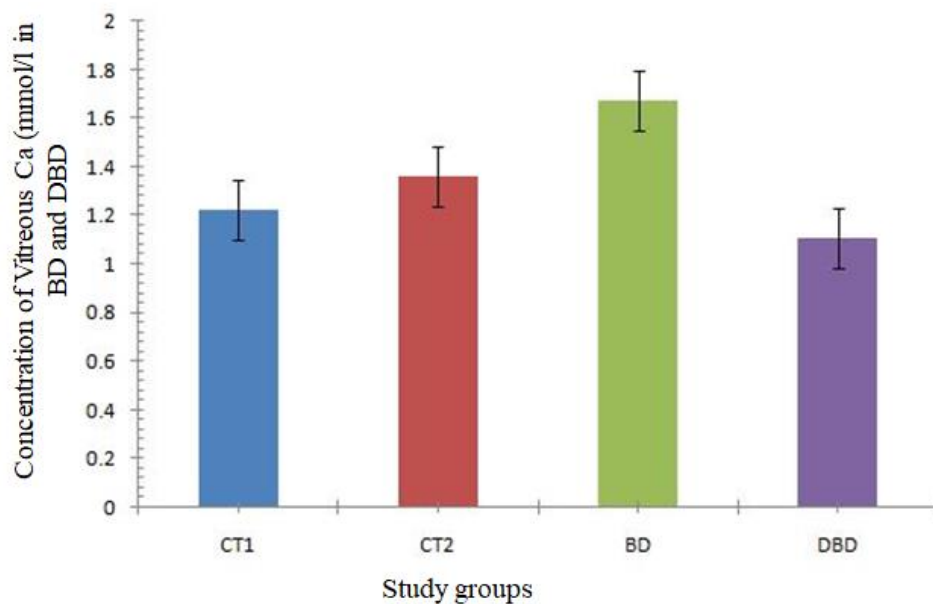


Fig. 5: Chart showing postmortem vitreous levels of Ca in BD and DBD. CT1: Control 1 (Strangulation death); CT2: Control 2 (Chloroform death); BD: Bleach administration death; DBD: Disguised bleach death (strangled to death before bleach administration)

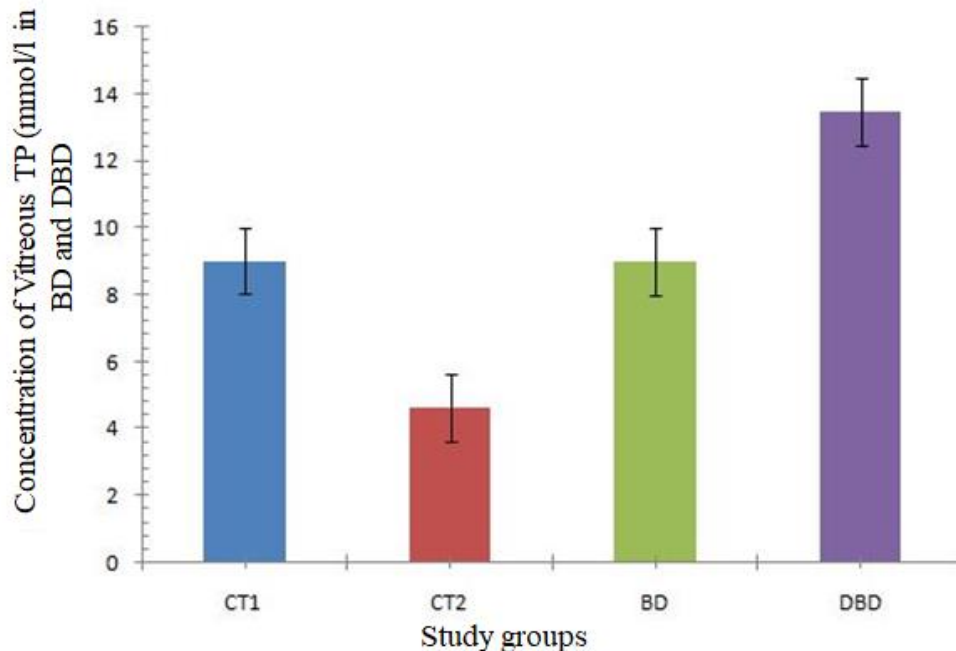


Fig. 6: Chart showing postmortem vitreous levels of total protein in BD and DBD. CT1: Control 1 (Strangulation death); CT2: Control 2 (Chloroform death); BD: Bleach administration death; DBD: Disguised bleach death (strangled to death before bleach administration); TP: Total protein

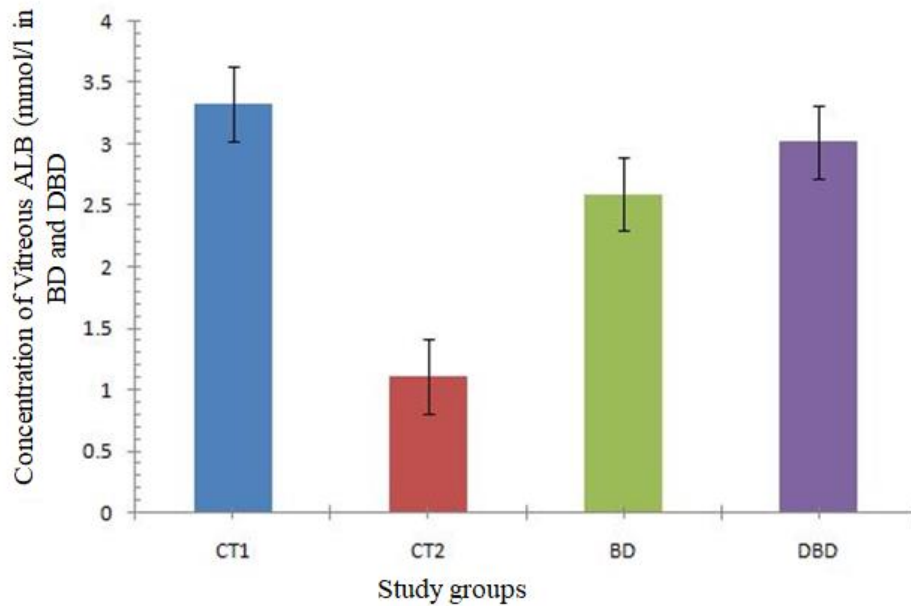


Fig. 7: Chart showing postmortem vitreous levels of albumin in BD and DBD. CT1: Control 1 (Strangulation death); CT2: Control 2 (Chloroform death); BD: Bleach administration death; DBD: Disguised bleach death (strangled to death before bleach administration); ALB: Albumin

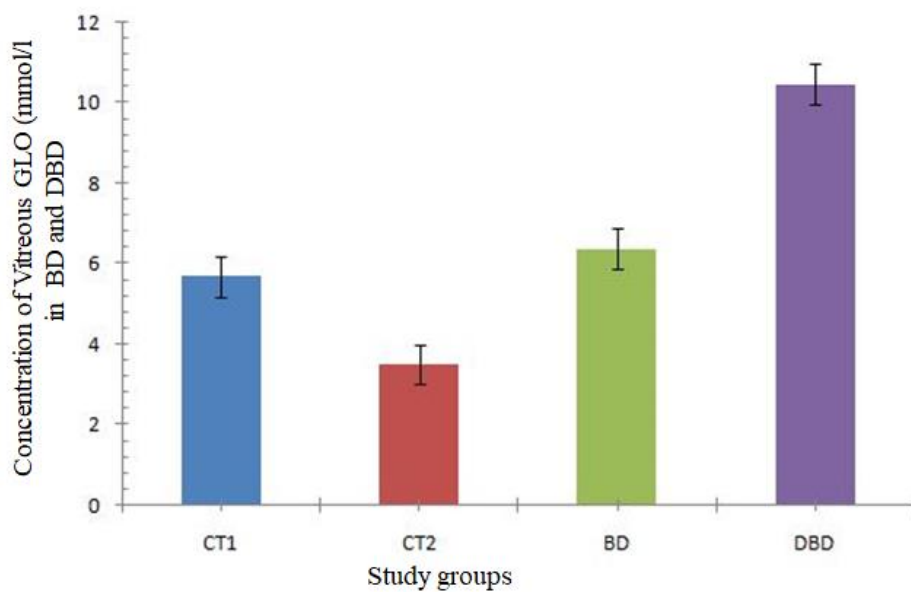


Fig. 8: Chart showing postmortem vitreous levels of globulin in BD and DBD. CT1: Control 1 (Strangulation death); CT2: Control 2 (Chloroform death); BD: Bleach administration death; DBD: Disguised bleach death (strangled to death before bleach administration); GLO: Globulin

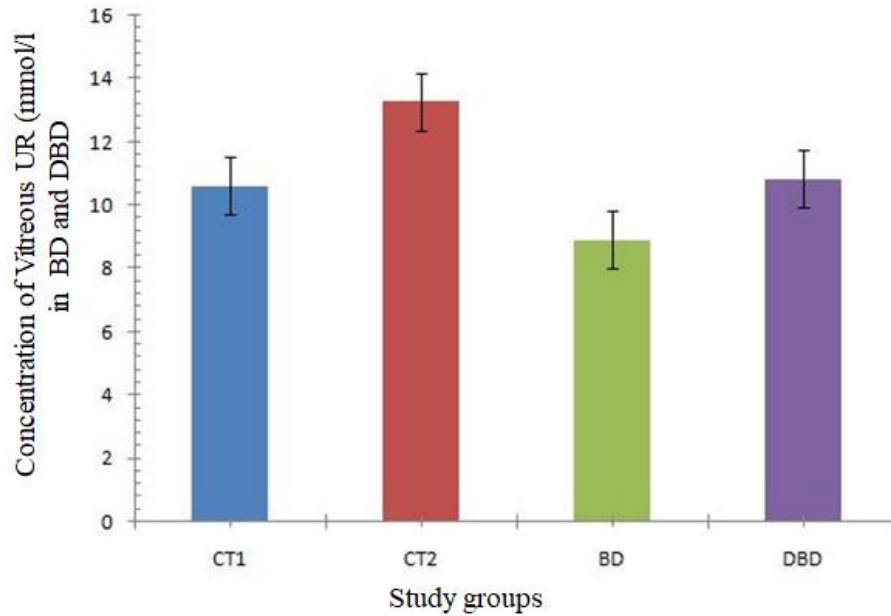


Fig. 9: Chart showing postmortem vitreous levels of urea in BD and DBD. CT1: Control 1 (Strangulation death); CT2: Control 2 (Chloroform death); BD: Bleach administration death; DBD: Disguised bleach death (strangled to death before bleach administration); UR: Urea

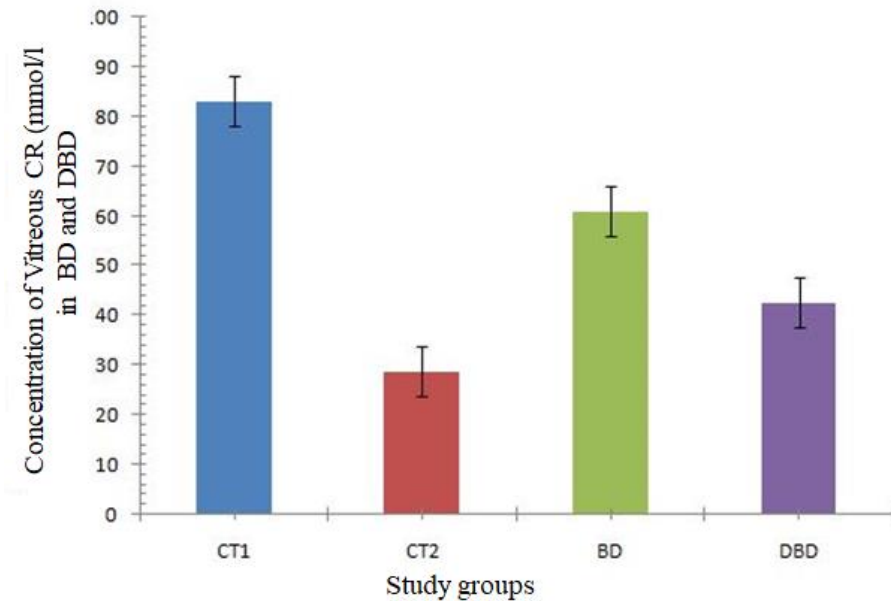


Fig. 10: Chart showing postmortem vitreous levels of creatinine in BD and DBD. CT1: Control 1 (Strangulation death); CT2: Control 2 (Chloroform death); BD: Bleach administration death; DBD: Disguised bleach death (strangled to death before bleach administration); CR: Creatinine

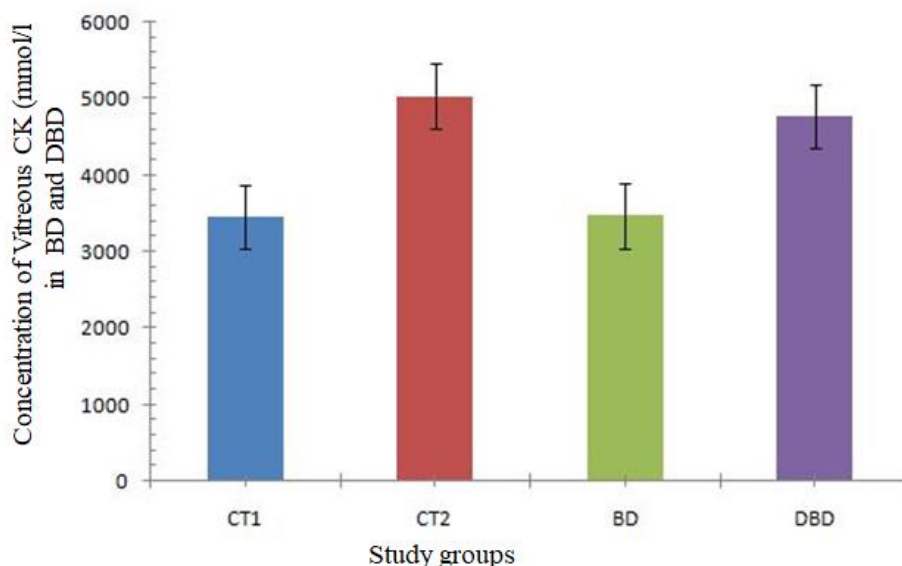


Fig. 11: Chart showing postmortem vitreous levels of creatine kinase in BD and DBD. CT1: Control 1 (Strangulation death); CT2: Control 2 (Chloroform death); BD: Bleach administration death; DBD: Disguised bleach death (strangled to death before bleach administration); CR: Creatine kinase

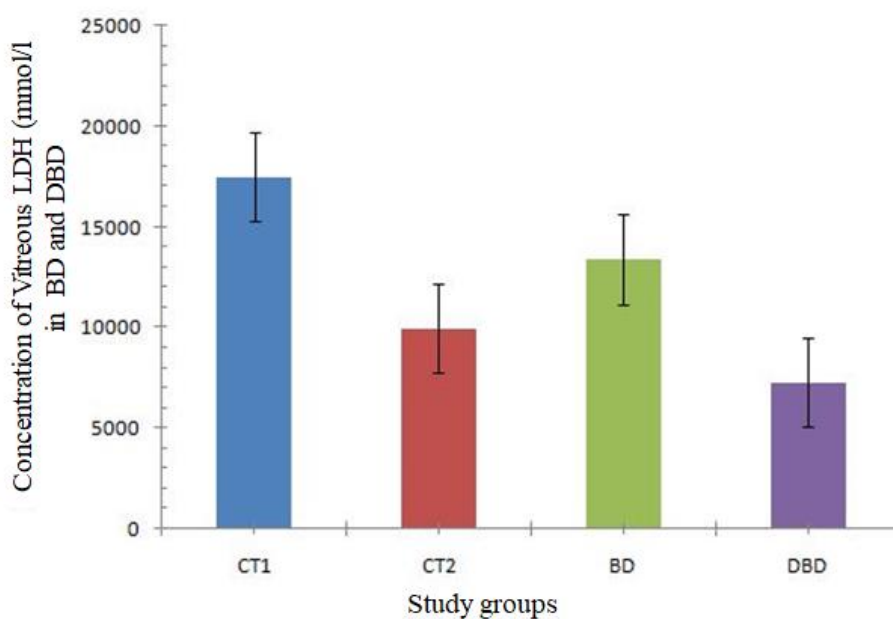


Fig. 12: Chart showing postmortem vitreous levels of lactate dehydrogenase in BD and DBD. CT1: Control 1 (Strangulation death); CT2: Control 2 (Chloroform death); BD: Bleach administration death; DBD: Disguised bleach death (strangled to death before bleach administration); LDH: Lactate dehydrogenase

The observed low level of Na in BD (94.000 ± 0.040825 mmol/l) when compared to DBD (157.000 ± 0.40825 mmol/l) and the respective controls (139.000 ± 0.40825 mmol/l) (145.000 ± 0.040825 mmol/l) can be attributed to the hyponatremia that is associated with acute kidney injury caused by the toxicity of sodium hypochlorite in bleach (Chung *et al.*, 2022). The observed low level of K in BD (21.900 ± 0.04082 mmol/l) when compared to DBD

(38.700 ± 0.04082 mmol/l) and control I (38.300 ± 0.04082 mmol/l) can be attributed to the hypokalemia associated with sodium hypochlorite generated tubular acidosis, where the kidney fails to excrete hydrogen ions leading to the sequestration of potassium ions in the urine (Vasudevan *et al.*, 2019; Zilg *et al.*, 2022).

The observed low level of Cl in BD (71.000 ± 0.040825 mmol/l) when compared to DBD

(105.000 ± 0.40825 mmol/l) and the respective controls (101.000 ± 0.47871 mmol/l) (99.000 ± 0.40825 mmol/l) can be attributed to effects of hypochloremia and dehydration that accompany excessive vomiting. Excessive vomiting is an established adverse response that is concomitant with sodium hypochlorite ingestion (Gwaltney-Brant, 2022; Khorasani et al., 2017). This outcome corroborates the findings of Zilg *et al.* (2016) which reported that postmortem chloride levels follow the same pattern as postmortem sodium levels.

The observed high levels of CO₂ in test groups (BD and DBD) respectively (14.000 ± 0.40825 mmol/l) (15.000 ± 0.40825 mmol/l) and in control 2 (16.000 ± 0.40825 mmol/l) when compared with control I (10.000 ± 0.47871 mmol/l) may be due to the fact that chlorine easily combines with carbon and carbon - containing compounds to form products that are eliminated from the body by decomposition to CO₂ (EPA, 2001). The observed levels of total protein in both the test and control groups are reflective of the patterns of globulin levels in all groups. This outcome agrees with the findings of Agoro *et al.*, 2017, who reported that globulin and total proteins levels were always consistent. In different studied groups, this consistency in globulin and total protein concentrations was also discovered in drowning studies (Agoro *et al.*, 2020). The observed high levels of total protein in the test groups (BD and DBD) respectively (8.974 ± 0.00041 mmol/l) (13.488 ± 0.0041 mmol/l) and control I (9.021 ± 0.00041 mmol/l) when compared with control 2 (4.613 ± 0.00041 mmol/l) can be ascribed to the tissue preserving properties of chloroform which prevents autolysis and makes tissue proteins unavailable for detection during analysis (Ruchieka *et al.*, 2014; Sacco *et al.*, 2022).

The observed higher levels of triacylglycerols in the test groups (BD and DBD) respectively (1.037 ± 0.000 mmol/l) (1.870 ± 0.19578 mmol/l) when compared with the controls

respectively (0.010 ± 0.0000 mmol/l) (0.311 ± 0.0000 mmol/l) can be linked with the deep tissue destruction connected with deaths caused by sodium hypochlorite which often results in the saponification of fats (ATSDR, 2014). The observed low level of creatinine in all the chlorine containing groups: test group (BD and DBD) (60.932 ± 0.00041 mcmol/l) (42.511 ± 0.00041 mcmol/l) and control 2 (28.663 ± 0.00041 mcmol/l) when compared with control I (83.002 ± 0.0041 mcmol/l) may be due to acute kidney injury that has become trite with chlorine medicated toxicity, which manifests in hematuria as blood and its contents pass into the urine (Peck *et al.*, 2011; Verma *et al.*, 2013).

The observed lower level of creatine kinase activity in BD (3468.853 ± 0.00041 U/L) when compared with control 2 (5035.685 ± 0.00041 U/L) can be traced to tissue disruption and protein modification characteristics of sodium hypochlorite as against the mild tissue integrity sustaining effects of chloroform (Peck *et al.*, 2011; Sacco *et al.*, 2022). The observed higher level of lactate dehydrogenase activity in control I (17491.880 ± 0.00408 U/L) when compared with BD (13390.890 ± 0.00479 U/L) suggests that the extent of oxygen deprivation and attendant ischemia caused by deaths by means of control I is greater than that caused by BD, because lactate dehydrogenase activity which is essentially an anaerobic process predominates in hypoxic circumstances. While the observed lower level of lactate dehydrogenase activity in DBD (7265.726 ± 0.00041 U/L) is indicative of sodium hypochlorite initiated postmortem changes (Farhana and Lappin, 2023; Rosato *et al.*, 2021; Verma *et al.*, 2013).

4.0 Conclusion

Postmortem vitreous levels of Na, K, Cl, total protein, creatinine and lactate dehydrogenase, differed significantly ($P \leq 0.05$) between samples from deaths by means of bleach toxicity and deaths by means of strangulation but disguised

as bleach toxicity. Postmortem vitreous levels of CO₂ and creatine kinase differed significantly ($P \leq 0.05$) between samples from deaths by means of bleach toxicity and deaths by means of chloroform toxicity. These identified analytical metabolites with discriminatory potentials between death by bleach toxicity and death by other means but disguised as bleach toxicity can be exploited as indicators in forensic investigations for the resolution cases where the cause of death is unclear and in contention of being either truly caused by bleach toxicity or suspected to be by other means but purported to be by bleach toxicity.

Authors contributions

Dr. C. G. Ikimi conceptualized the work, did the laboratory analysis and wrote the manuscript. Mrs. U. O. Abosede conducted the sample collection. Dr. J. C. Ifenkwe did the statistical analysis. All authors read and approved the manuscript.

Ethical Approval

Ethical clearance was obtained from the animal research ethics committee of the Nnamdi Azikiwe University, Awka. The Animal Welfare Act of 1985 of the United States of America for research and Institutional Animal Care and Use Committee (IACUC) protocols were stringently adhered to (Benjamin and Jean, 2016).

Conflict of interest

The authors declared no conflict of interests.

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